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THE EFFECT OF LIGNIN AND BARK WOUNDING ON SUSCEPTIBILITY OF SPRUCE TREES TO *Dendroctonus micans*

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Abstract—The effect of lignified stone cell masses (lignin) and mechanical wounding of bark on gallery formation and oviposition by the spruce bark beetle, *Dendroctonus micans*, was determined in plantations of Norway and Sitka spruce. When beetles were implanted onto trees that varied in bark lignin concentration, there was a significant negative relationship between lignin and adult gallery size. Only a few larval galleries were established, all of them on trees with a low lignin concentration. Results confirm the importance of lignin as a preformed defence in living trees. Adults excavated significantly larger galleries in wounded than unwounded bark. Most larval galleries were also established in wounded bark. The concentrations of nitrogen, carbohydrate, and resin and the moisture content of wounded and unwounded bark were measured at the beginning of the experiment. A number of significant changes were induced by wounding, including an increase in the concentration of nitrogen and starch, and decreases in the moisture content and the concentration of free sugars. There was no overall effect of wounding on resin content of bark, although concentrations were significantly lower in new than old wounds. An increase in the nutritional quality of bark following wounding appears to be the main factor influencing attacks on wounded trees by *D. micans*.

Key Words—Spruce, lignin, nutrients, starch, defense, resin.

INTRODUCTION

The spruce bark beetle, *Dendroctonus micans* (Kug.) (Coleoptera: Scolytidae), in contrast to most other economically important bark beetles (Coulson, 1979; Horntvedt et al., 1983; Raffa and Berryman, 1983), is not dependent on adult

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mass attack and subsequent tree death for successful development of its larvae (Grégoire, 1988). This solitary species infests individual trees within a forest stand without necessarily killing them, at least in the short term. The factors that influence the distribution of attack within the stand as well as the fate of attacked trees are of considerable significance in the management of this primary pest.

Host resistance plays a central role in the population dynamics of mass-attack bark beetles and can influence the distribution of attack within forests (Vité and Wood, 1961; Raffa and Berryman, 1987; Reid et al., 1967; Berryman, 1969; Christiansen and Horntvedt, 1983). In conifers, resin is one of the principal defenses against bark beetle attack, especially in pines (Vité and Wood, 1961; Hodges et al., 1979; Hain et al., 1985; Raffa and Berryman, 1987). In spruce, resin flow is generally less copious than in pines (Christiansen and Horntvedt, 1983), and *D. micans* appears to have a relatively high tolerance of the resin and its constituent monoterpenes (Everaerts et al., 1988). Lignified stone cell masses, which are abundant in the bark of some spruce trees but absent in pines, appear, from the results of laboratory experiments, to be an important preformed physical defense against both adults and larvae of *D. micans* (Wainhouse et al., 1990), although their effects have not been directly tested on living trees.

The distribution of attack by *D. micans* within stands may also be affected by the occurrence of mechanical damage to bark caused, for example, by falling trees or timber extraction (Chararas, 1960; Evans et al., 1984). The association between bark wounding and attack by *D. micans* could simply reflect the attraction of beetles to monoterpenes in spruce resin exuding from the wounds (Vasechko, 1978; Wainhouse et al., 1992). On the other hand, wounding may induce changes in bark that favor oviposition and larval survival and development.

In this paper, we report the results of experiments on Norway and Sitka spruce to determine the effect of preformed lignified stone cell masses on adult gallery formation and oviposition. We also report on changes in the nutritive and defensive status of bark induced by mechanical wounding.

METHODS AND MATERIALS

Trees were selected within adjacent compartments of Norway [*Picea abies* (L.) Kar.] and Sitka spruce [*P. sitchensis* (Bong.) Carr.] at the Long Mynd, Shropshire (British National Grid ref. SO 412 902), planted in 1950–1952. The trees were unwounded and free from attack by naturally occurring *D. micans*.

Origin of Adults and Implantation. Beetles were collected either from naturally attacked forest trees or from Norway spruce logs implanted with second

instars. Individual adults, weighing more than 29 mg and therefore assumed to be females (Robinson et al., 1984), were confined under a 15-mm-diameter plastic cap on an area of bark from which a 5-mm-diameter core (to the cambium) had been removed. Following implantation, the trunks were loosely enclosed in Tygan netting to prevent predation by woodpeckers (Picidae).

Lignified Stone Cell Masses. Eight trees, 16–27.5 cm in diameter at 1.3 m [diameter at breast height (dbh)], were selected in each compartment, four with a high and four with a low concentration of lignified stone cell masses (lignin) in bark, based on visual assessment (Wainhouse and Ashburner, 1996). Selection ensured a range of lignin concentrations among experimental trees. Beetles were implanted on trees in October 1989 at 75, 125, and 175 cm above ground. Three beetles were used at each height, spaced equally around the circumference from the north-facing side of the tree.

At assessment the following August, the occurrence of living or dead beetles was recorded and, after removing the outer bark, a tracing was made of the gallery area excavated by adults or larvae. This area did not include the bark affected by the dynamic wound response (Reid et al., 1967; Berryman, 1969; Raffa and Berryman, 1987), which had formed around most galleries by the time of assessment. Gallery area in this and the wounding experiment, was measured by a PC-based image analysis system. Two transverse sections of bark were removed at each height for a visual estimation of percentage lignin (Wainhouse and Ashburner, 1996).

Bark Wounding. For both Norway and Sitka spruce, 18 trees (15–28.3 cm dbh) with a low lignin concentration at bh were wounded on three occasions. Wounds made at different times were randomly assigned to one of three heights (centered at 90, 170, or 250 cm) and four cardinal points of the compass. Wounds were made by removing, to the cambium, 32 cores of bark 1.5 cm diam. and approximately 6 cm apart (Figure 1). This pattern was selected because it circumscribed an area of intact bark through which beetles could tunnel while remaining close to a wound.

Only two of the three wounds made on each tree were used in the experiment because of limited availability of beetles. The wounds used were made either 11 months or 8–10 days before the start of the experiment (September 26–October 10, 1989), at which time an adult was implanted into the center of each wounded area (Figure 1) with a control implantation at a corresponding position on the opposite, unwounded side of the tree. Gallery formation and oviposition were assessed in August 1990. Living or dead beetles were recorded and, after removing the outer bark, a tracing was obtained of the gallery area excavated by each adult or larvae and adult. Larvae were collected for later counting and instar determination.

At the time of beetle implantation, four 1.5-cm-diameter bark cores were taken from the wounded (Figure 1) and unwounded sides of the tree for deter-

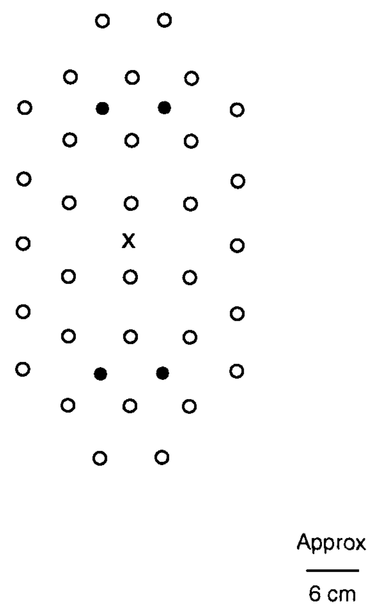

FIG. 1. Pattern of wounding with 1.5-cm-diam. cores (○) and position of samples for chemical analysis (●) and beetle implantation (×).

mination of nitrogen, carbohydrate, and resin concentration. The cores were from fresh bark not affected by the small necrotic area that formed around the circumference of each circular wound. Cores were placed on Dry Ice after sampling before storage at −55°C. Before processing, lichens and loose bark were brushed from the outer surface of cores and in the few samples, where a thick rhytidome was present, this was also removed. Fresh material was used for gravimetric determination of resin content as outlined in Wainhouse et al. (1997). Carbohydrate and nitrogen analysis was on pooled samples dried at 70°C to constant weight. Samples were ground in a rotor-speed mill (0.5-mm sieve perforations) prior to digestion in a sulfuric acid–hydrogen peroxide mixture (Wolf, 1982) to produce a clear colorless solution. Total nitrogen was determined colorimetrically as ammonia by the reaction with salicylate and dichloroisocyanurate using nitroprusside as catalyst. The method employs eight calibration standards and certified reference material was run in every batch of analyses. Total sugar, free sugar, and starch content were determined by the method outlined in Ward and Deans (1993). Moisture content was determined from fresh samples dried to constant weight at 70°C.

Statistical Analysis. Data were analyzed using SAS, Genstat, and CSS

statistical packages. Data on percent lignin and adult gallery size were analyzed by regression. For the wounding experiment, exclusion of some trees that suffered additional natural attack and the failure of some beetles to become established resulted in an unbalanced design and data were analyzed using the SAS "mixed" procedure. For each variate (gallery area and percentage nitrogen, starch, free sugars, total sugars, resin, and moisture content), an initial analysis was done using the "full" model of all factors (species, wound, and age of wound) and interactions and including possible covariates. Transformations were applied as appropriate based on plots of residuals to achieve approximately normal distributions. The final model used included only those factors, interactions, and covariates that were significant ($P < 0.05$) or near significant in the initial analysis. Percent lignin was used as a covariate in all analyses except for gallery area, starch, and resin, with adult weight a covariate only for the analysis of gallery area (Table 1).

RESULTS

Lignified Stone Cell Masses. In this and the bark wounding experiment 8–15% of beetles died without initiating galleries. Approximately 60% of beetles

TABLE 1. MEAN ADULT GALLERY SIZE AND BARK CHARACTERISTICS ESTIMATED BY MULTIVARIATE ANOVAR OF DATA FROM WOUNDING EXPERIMENT[a]

	Wound					
	New	Old	P_1	Control (mean)	P_2	Covariate
ln gallery area (mm^2)	6.07	5.76	NS	5.62	*	adult weight (+)
Nitrogen (%)[b]	0.58	0.62	**	0.55	***	percent lignin (−)
Starch (%)	0.67	1.29	***	0.29	***	
Free sugars (%)	7.19	8.18	***	8.21	**	percent lignin (−)
Total sugars (%)	7.73	9.36	***	8.25	NS	percent lignin (−)
Resin (%)[c]	1.79	2.25	**	1.99	NS	
Moisture (%)[b]	54.0	52.0	***	54.5	***	percent lignin (−)

[a] Significance of difference between new and old wounds (P_1) and wounded (new + old) and control (P_2). Means are predicted from the model (see text) and adjusted for covariate where appropriate. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

[b] Significant species effects were found, figures presented are overall mean response to wounding and control. Species means are given in Table 3.

[c] Means are back-transformed from % resin$^{-0.85}$ used in the analysis (see text).

that established galleries were dead at assessment, with 40% missing, presumably through reemergence and dispersal. Beetle mortality was not related to lignin concentration, and there were no differences in mortality between the tree species. On Norway spruce, two living beetles were recovered. Mean adult gallery area (log(ln) transformed) was determined for each tree and used in a regression on tree mean percent lignin (Figure 2). Results for both Norway and Sitka spruce were similar with, overall, a significant negative relationship between gallery area and percent lignin ($R^2 = 0.30$, $P < 0.05$).

In a total of five galleries on two Norway spruce trees and a single gallery on Sitka spruce, oviposition resulted in larval establishment. On all three trees, lignin concentration was relatively low (Figure 2). The resulting larval galleries were not included in the regression analysis.

Bark Wounding. Within galleries, approximately 30% of beetles were dead and 5% alive at assessment, with the remaining beetles missing. On some trees, more than one adult gallery was found within a given wounded area or its equivalent on the unwounded side. Some of these galleries appeared to result from second attacks by the original implanted beetle and were therefore included in the total gallery area. On the unwounded side of two trees, beetles reemerged and initiated galleries close to "wounds" made by taking cores for chemical analysis. These galleries were excluded from the data analysis, as were two Sitka spruce trees that were attacked by naturally occurring beetles.

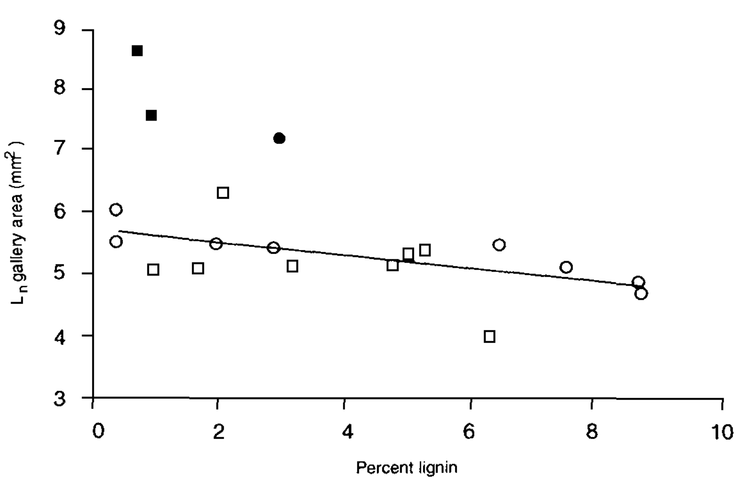

FIG. 2. Relationship between the mean size of galleries formed by adult female *D. micans* on Norway (□) and Sitka spruce (○) and percent lignin in bark, $y = 5.73 - 0.11x$. The mean size of larval galleries on Norway (■) and Sitka spruce (●) is also shown but was not included in the regression.

The main analysis was based on seven variates: log (ln) adult gallery size, percent resin$^{-0.85}$, percent nitrogen, percent moisture content, and percent total sugars, free sugars, and starch. For percent lignin, the power transformation was selected on the basis of a near linear normal probability plot. Mean values, adjusted for covariates, with appropriate significance levels for treatment effects were those predicted from a balanced model (Table 1). Adult galleries were significantly larger within wounded bark (new + old), but there was no significant effect of wound age on gallery size. Larvae became established on four Sitka and nine Norway spruce trees. Three of the larval galleries on Norway spruce contained dead larvae within resin-soaked frass. The remaining galleries contained living larvae whose developmental stage suggested that egg hatch and larval feeding had not commenced until the spring following implantation of the beetles in the previous autumn. There were too few larval galleries for inclusion in the main analysis but summary data, combining species and wound age, are shown in Table 2. These data are consistent with those from adult galleries, indicating a tendency for larvae to form galleries more readily within wounded bark and for more of them to survive than in unwounded bark.

Wounding significantly increased the percentage nitrogen and starch content of bark, with the highest concentrations on the old wounds (Table 1). The concentrations of total sugars and resin were also significantly higher in the old wounds, but there was no overall effect of wounding. The lowest concentration of free sugars was found on new wounds and the lowest moisture content on old wounds. Only two of the variables measured differed significantly between tree species. Norway spruce bark had a higher nitrogen content than that of Sitka spruce, whereas moisture content was higher in Sitka spruce bark (Table 3).

DISCUSSION

In both experiments, 30–40% of beetles died after some initial tunneling within the bark. Few beetles laid eggs and most appeared to redisperse, suggesting that some of the trees were relatively resistant to attack. This conclusion

TABLE 2. LARVAL GALLERIES DEVELOPING ON WOUNDED AND UNWOUNDED BARK[a]

	Wounded	Unwounded
No. of galleries	15	4
Larvae/gallery	123.8 ± 15.2 (12)	84.5 (2)

[a] Data combined for Norway and Sitka spruce and for new and old wounds (see text). Means based on (*N*) observations ± SE.

TABLE 3. SPECIES MEANS FOR PERCENT NITROGEN AND MOISTURE CONTENT

Species	Nitrogen (%)	P^a	Moisture (%)	P^a
Norway	0.60	<0.05	52.1	<0.001
Sitka	0.55		55.4	

[a]Significance of difference between species.

supports earlier observations of unattacked trees within heavily infested stands and the widespread occurrence of abortive attacks (Bevan and King, 1983). Part of that resistance can be attributed to the presence of lignin in bark, which can affect beetle tunneling and oviposition (Wainhouse et al., 1990). Variation in lignin concentration both between and within trees (Wainhouse and Ashburner, 1996; Wainhouse et al., 1997) seems likely to have a significant effect on the distribution and success of attack within spruce forests.

The effects of lignin were also evident in the wounding experiment. Although low lignin trees were selected in order to minimize effects on beetles, there remained sufficient variation in lignin content both within and between trees for it to be a significant covariate for several of the variables analyzed (Table 1). The negative correlation between lignin and both nitrogen and starch content probably reflects the reduced proportion of living tissue in bark with a high lignin content. This could enhance any direct toxic or antifeedant property of lignin, increasing its effectiveness as a defense against *D. micans*.

Wounding itself induced changes in adjacent intact bark that were long lasting and had positive effects on adult gallery size and larval establishment. Probably the most important change was the increase in concentration of both starch and nitrogen, which was higher in the old (11 months) than the new wounds (8–10 days). In previous studies, changes in the starch content of bark following removal of bark strips have been related to the degree to which the wounds prevented downward movement of carbohydrate from sources in the tree crown and upper bole (Miller and Berryman, 1986). The wounding pattern used in our experiments would form a partial barrier to phloem transport but starch content increased rather than decreased compared to control areas. This suggests that the changes were not a passive effect of isolation from the upper crown. The amount of starch in bark has been linked to defense against bark-invading organisms. While starch reserves may (Waring and Pitman, 1980, 1985; Larsson et al., 1983; Lorio and Hodges, 1985; Wright et al., 1979) or may not (Christiansen and Ericsson, 1986) provide a reliable index of resistance, they do at least indicate the potential to respond to infection. This arises because starch is metabolized during a defense reaction (Reid et al., 1967; Christiansen

and Ericsson, 1986), reflecting the often high energy requirements of secondary chemical production (Gershenzon, 1994). The changes caused by wounding in our experiments appear, therefore, to have been an active response that seems likely to have increased the potential for a strong induced defensive reaction. The increase in nitrogen concentration may be related to active repair processes following wounding of bark (Mullick, 1977; Biggs et al., 1984) and is likely to have enhanced the nutritional value of bark for beetles.

Wounding bark reduced the moisture content, as has also been reported for damaged foliage (Hartley and Lawton, 1987). Likely effects of changes in moisture content on *D. micans* are difficult to assess because beneficial effects on larval survival or development have been reported for both low (Berryman, 1972; Wagner et al., 1979; Webb and Franklin, 1978) and high bark moisture content (Storer and Speight, 1996). We would expect nitrogen concentration to be a major determinant of bark nutritional quality and, in our study, it was negatively related to moisture content of wounded bark. In Norway spruce, the preferred host of *D. micans*, nitrogen concentration was higher and moisture content lower than in Sitka spruce.

Changes in the resin content of bark induced by wounding were difficult to interpret in terms of defense. There appears to be an initial decrease in concentration shortly after wounding, but in the 11-month-old wounds concentrations were higher than in unwounded bark. *D. micans* has a high tolerance for resin (Everaerts et al., 1988) and we conclude that the major effect of wounding spruce bark is to increase the nutritive quality of surrounding bark for this bark beetle.

The effect of wounding spruce bark contrasts markedly with that reported for leaves from a number of mainly broad-leaved tree species where damage commonly results in an increase in defensive chemicals and a decrease in nutritive quality (Smith, 1988; Haukioja, 1990). The effects of wounding on bark may be an unavoidable consequence of the need to repair damaged tissue and to maintain an adequate reservoir of carbohydrates to fuel induced defensive responses.

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